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PHENOL RED-CHINA BLUE AS AN INDICATOR IN FERMENTATION TESTS OF BACTERIAL CULTURES

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A special medium for the differentiation of members of the typhoid-coli group of bacteria was devised by me[1] in 1916; it contained decolorized china blue as an indicator. The use of this stain in combination with malachite green had been recommended in 1911 by Bitter[2] in a special medium for the isolation of typhoid bacilli from stools. Harding and Ostenberg[3] employed decolorized "soluble blue," a stain related to china blue, as an indicator for acid formation in broth cultures. Bronfenbrenner[4] recommended the use of a combination of china blue and rosolic acid as an indicator for both acid and alkali in fluid mediums for fermentation tests of growing bacterial cultures. He did not decolorize the china blue before adding it to the medium; but if the medium was sufficiently alkaline, the china blue was decolorized during the sterilization. Bronfenbrenner, Davis, and Morishima[5] described the action of this indicator in milk mediums. The fact that rosolic acid is insoluble in water appeared to me to be a disadvantage.

The indicator which I herewith recommend is composed of phenol red and decolorized china blue. A 1% solution of china blue in distilled water is prepared and, while the solution is kept hot on a water bath, normal sodium hydroxid solution is added slowly until the blue color disappears and the solution becomes brown. This is the decolorized china blue solution. It should be kept in a flask with a rubber stopper. About 3.5 c c of normal sodium hydroxid are required to decolorize 100 c c of the 1% china blue solution. To the peptone solution, sugar-free broth or other fluid medium to be used, are added 5 c c of 0.02% solution of phenol red per 100 c c of medium, and the reaction is adjusted to the desired P_H. For the typhoid-coli group I have used $P_H = 7.0$ to 7.2. Then 1.2 c c of the decolorized china blue solution is added to every 100 c c of medium, and sterilization is carried out in the autoclave. The sugar solution can be sterilized separately and then added to the sterilized medium containing the

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[1] Jour. Bacteriol., 1916, 3, p. 19.
[2] München. med. Wchnschr., 1911, 58, p. 709.
[3] Jour. Infect. Dis., 1906, 2, p. 109.
[4] Jour. Med. Research, 1919, 39, p. 25.
[5] Ibid., p. 345.

indicators, when the tubing must, of course, be performed under aseptic conditions; or, the sugar can be added to the previously sterilized medium, and after being tubed, this can be heated a second time in the autoclave—this time, however, for only 10 minutes and at 10 lbs. pressure. If properly prepared, the medium is almost colorless; if, however, after sterilization, it is tinged with color, it can still be satisfactorily employed by carrying a control uninoculated tube with every set of fermentation tests. The control tube should in any case never be omitted as the mediums during incubation, for longer periods of time may absorb carbon dioxid and become more acid. After inoculation and incubation of the medium the production of alkali during bacterial growth is indicated by a pink color due to the phenol red, the china blue remaining colorless. The production of acid causes first a bright green color, due to the yellow of the phenol red plus the blue of the china blue; later, when much acid is formed, the green color changes to a deep blue. The color changes are much sharper, and consequently more easily and accurately read than those due to litmus.

I have also employed the decolorized china blue as an indicator in agar instead of the decolorized fuchsin of Endo. The nutrient agar is adjusted to the neutral point of litmus or to $P_H = 7.0$ to 7.2, the sugar is added, and 1.2 c c of the decolorized china blue per 100 c c of agar.

On plates of this medium colonies fermenting the contained sugar take on a deep blue color, while the nonfermenters remain white or pale blue. The decolorized china blue plates possess a distinct advantage over the endo-plates, in that they do not change color on exposure to light as do the latter.

Both the fluid and solid mediums described above have been used with very satisfactory results by Dr. Teague and myself in a large series of tests in connection with some experimental work on typhoid and paratyphoid bacilli.